

CLAIMS:

1. A method for determining the modification conditions of a therapeutic agent to prevent host-mediated inactivation of said therapeutic agent comprising

- (1) assaying the biological activity of a first modified therapeutic agent after said first modified therapeutic agent has been administered to a subject;
- (2) assaying the biological activity of said first modified therapeutic agent after at least one booster dose of said first modified therapeutic agent has been administered to said subject;
- (3) carrying out (1) and (2) with an additional modified therapeutic agent that has been modified differently than said first modified therapeutic agent; and
- (4) comparing the biological activity of said first modified therapeutic agent with the biological activity of said additional modified therapeutic agent.

2. The method of claim 1, wherein said additional modified therapeutic agent is modified with the same modifying agent as said first modified therapeutic agent.

3. The method of claim 2, wherein said modifying agent is polyethylene glycol (PEG).

4. The method of claim 3, wherein said PEG is selected from the group consisting of mono-methoxy succinimidyl butanoate (SBA)-PEG, succinimidyl carbonate (SC)-PEG, aldehyde (ALD)-PEG, and succinimidyl propionate (SPA)-PEG.

5. The method of claim 1, wherein said additional modified therapeutic agent is modified to the same extent as said first modified therapeutic agent.

6. The method of claim 1, wherein said additional modified therapeutic agent and first modified therapeutic agent are modified with different modifying agents.

5 7. The method of claim 1, wherein said therapeutic agent is a polypeptide.

8. The method of claim 7, wherein said polypeptide is used to treat viral infections in patients in need of treatment thereof.

9. The method of claim 7, wherein said polypeptide is used to treat cancer in patients in need of treatment thereof.

10 10. The method of claim 7, wherein said polypeptide has a monomeric molecular weight of about 300 daltons to about 300,000 daltons.

11. The method of claim 7, wherein said polypeptide is used to lower glutamine levels in a subject.

12. The method of claim 7, wherein said polypeptide is used to lower asparagine levels in a subject.

13. The method of claim 7, wherein said polypeptide is used to lower asparagine and glutamine levels in a subject.

14. The method of claim 1, wherein said therapeutic agent is a nucleic acid.

15. The method of claim 14, wherein said nucleic acid is used to treat a viral infection in patients in need of treatment thereof.

16. The method of claim 14, wherein said nucleic acid is used to treat cancer in patients in need of treatment thereof.

5 17. A method of preparing a pharmaceutical composition where host-mediated inactivation is prevented, comprising ascertaining the modification conditions of a therapeutic agent by the method of claim 1 and modifying said therapeutic agent according to said modification conditions.

10 18. The method of claim 17, wherein said pharmaceutical composition further comprises an excipient.

19. The method of claim 18, wherein said excipient protects said therapeutic agent during lyophilization.

20. The method of claim 17, wherein said therapeutic agent comprises glutaminase-asparaginase.

15 21. The method of claim 20, wherein said therapeutic agent is *Pseudomonas* glutaminase-asparaginase.

22. The method of claim 21, wherein said *Pseudomonas* glutaminase-asparaginase is modified with polyethylene glycol.

20 23. The pharmaceutical composition prepared by the method of claim 17, wherein said pharmaceutical composition comprises a glutaminase-asparaginase that has been modified

with succinimidyl carbonate polyethylene glycol 5000 (SC-PEG 5000), wherein said glutaminase-asparaginase is modified to an extent of from about 21% to about 49% by SC-PEG 5000, and wherein said composition prevents host-mediated inactivation.

24. The composition of claim 23, wherein said glutaminase-asparaginase is modified
5 from about 26% to about 36% by SC-PEG 5000.

25. The composition of claim 24, wherein said glutaminase-asparaginase is modified about 31% by SC-PEG 5000.

26. The pharmaceutical composition prepared by the method of claim 17, wherein said pharmaceutical composition comprises a glutaminase-asparaginase that has been modified with mono-methoxy succinimidyl butanoate polyethylene glycol 5000 (SBA-PEG 5000), wherein said glutaminase-asparaginase is modified from about 25% to about 58% by SBA-PEG 5000, and wherein said composition prevents host-mediated inactivation.

27. The composition of claim 26, wherein said glutaminase-asparaginase is modified from about 30% to about 40% by SBA-PEG 5000.

15 28. The composition of claim 27, wherein said glutaminase-asparaginase is modified about 35% by SBA-PEG 5000.

29. The pharmaceutical composition prepared by the method of claim 17, wherein said pharmaceutical composition comprises a glutaminase-asparaginase that has been modified with aldehyde polyethylene glycol 2000 (ALD-PEG 2000), wherein said glutaminase-
20 asparaginase is modified from about 45% to about 65% by ALD-PEG 2000, and wherein said composition prevents host-mediated inactivation.

30. The pharmaceutical composition prepared by the method of claim 17, wherein said pharmaceutical composition comprises a glutaminase-asparaginase that has been modified with succinimidyl propionate polyethylene glycol 5000 (SPA-PEG 5000), wherein said modified glutaminase-asparaginase is modified from about 25% to about 65% by SPA-PEG 5000, and
5 wherein said composition prevents host-mediated inactivation.

31. The composition of claim 30, wherein said glutaminase-asparaginase is modified from about 40% to about 55% by SPA-PEG 5000.

32. A composition comprising a glutaminase-asparaginase, wherein said glutaminase-asparaginase has been modified with succinimidyl carbonate polyethylene glycol 5000 (SC-PEG 5000) to an extent of about between 21% and 49%.

33. The modified therapeutic composition of claim 32, wherein said glutaminase-asparaginase has been modified to an extent of about between 26% and 36%.

34. The modified therapeutic composition of claim 33, wherein said glutaminase-asparaginase has been modified to an extent of about 31%.

35. A composition comprising a glutaminase-asparaginase, wherein said glutaminase-asparaginase has been modified with succinimidyl butanoate polyethylene glycol 5000 (SBA-PEG 5000) to an extent of about between 25% and 58%.

36. The modified therapeutic composition of claim 35, wherein said glutaminase-asparaginase has been modified to an extent of about 30% to 40%.

37. The modified therapeutic composition of claim 36, wherein said glutaminase-asparaginase has been modified to an extent of about 35%.

38. A composition comprising a glutaminase-asparaginase, wherein said glutaminase-asparaginase has been modified with aldehyde polyethylene glycol 2000 (ALD-PEG 2000) to an
5 extent of about between 45% and 65%.

39. A composition comprising a glutaminase-asparaginase, wherein said glutaminase-asparaginase has been modified with succinimidyl propionate polyethylene glycol 5000 (SPA-PEG 5000) to an extent of about between 25% and 65%.

40. The modified therapeutic composition of claim 39, wherein said glutaminase-asparaginase has been modified to an extent of about 40% to 55%.

FOOTNOTES